AD A069333

DDC FILE COPY

MIDWEST RESEARCH INSTITUTE

REPORT

AD_____

MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS PHASE I: ACUTE ORAL TOXICITY, PRIMARY SKIN AND EYE IRRITATION, DERMAL SENSITIZATION, DISPOSITION AND METABOLISM, AND AMES TESTS OF ADDITIONAL COMPOUNDS

DDC
RECEIVED
JUN 4 1979
A

PROGRESS REPORT NO. 6
December 8, 1978

Contract No. DAMD-17-74-C-4073
MRI Project No. 3900-B

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

For

Contract Officer's Technical Representative: Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research and
Development Laboratory
Fort Detrick, Frederick, Maryland 21701

Animal Experimentation: Animal experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" (1974) prepared by the Institute of Laboratory Animal Resources, National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 91-570, "Laboratory Animal Welfare Act," 1970.

Disclaimer: The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

AD_____

MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS PHASE I: ACUTE ORAL TOXICITY, PRIMARY SKIN AND EYE IRRITATION, DERMAL SENSITIZATION, DISPOSITION AND METABOLISM, AND AMES TESTS OF ADDITIONAL COMPOUNDS

PROGRESS REPORT NO. 6
December 8, 1978

By

Harry V. Ellis, III
John R. Hodgson
Shang W. Hwang
Laurel M. Halpap
Danny O. Kelton
Bruce S. Andersen
Daniel L. VanGoethem
Cheng-Chun Lee

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD-17-74-C-4073
MRI Project No. 3900-B

Contract Officer's Technical Representative: Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research and
Development Laboratory
Fort Detrick, Frederick, Maryland 21701

Distribution Unlimited

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Progress Report No. 6

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): Mammalian Toxicity of Munitions Compounds, Phase I. Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization, Disposition and Metabolism and Ames Tests of Additional Compounds

5. TYPE OF REPORT & PERIOD COVERED: Progress Report, Feb. 1, 1975 - Feb. 28, 1978

6. PERFORMING ORG. REPORT NUMBER: MRI Project No. 3900-B

7. AUTHOR(s): Harry V. Ellis, III, John R. Hodgson, Shang W. Hwang, Laurel M. Halpap, Danny O. Helton, Bruce S. Andersen, Daniel L. VanGoethem, Cheng Chu Lee

8. CONTRACT OR GRANT NUMBER(s): DAMD 17-74-C-4073

9. PERFORMING ORGANIZATION NAME AND ADDRESS: Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 62720A 3A762720A835; 62758A 3A762758A835

11. CONTROLLING OFFICE NAME AND ADDRESS: U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, Maryland 21701

12. REPORT DATE: December 1978

13. NUMBER OF PAGES: 50

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, Maryland 21701

15. SECURITY CLASS. (of this report): Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report): Distribution unlimited

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES: Progress rept. no. 6, 1 Feb 75-28 Feb 78.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Trinitrotoluene (TNT; 2-Methyl-1,3,5-trinitrobenzene, CAS Reg. No. 118-96-7)
2,3-Dinitrotoluene (1-Methyl-2,3-dinitrobenzene, CAS Reg. No. 602-01-7)
2,4-Dinitrotoluene (1-Methyl-2,4-dinitrobenzene, CAS Reg. No. 121-14-2)
2,5-Dinitrotoluene (2-Methyl-1,4-dinitrobenzene, CAS Reg. No. 619-15-8)
2,6-Dinitrotoluene (2-Methyl-1,3-dinitrobenzene, CAS Reg. No. 606-20-2)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report supplements Progress Report No. 1, dated July 22, 1975. 3,5-Dinitrotoluene (3,5-DNT) was the most potent of all DNT isomers in oral acute doses to rats and mice. 2-Amino-4,6-DNT (2-ADNT) and its isomer, 4-ADNT, were the least potent in rats and female mice, and comparable to 2,3-DNT and 2,4-DNT in male mice. 3,5-DNT and 4-ADNT were not irritating to rabbit skin; 2-ADNT was a mild irritant. All three compounds were not irritating to rabbit eyes and not sensitizing to guinea pigs. Tests indicated that

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

230 350

9 (concluded)

),4-Dinitrotoluene (4-Methyl-1,2-dinitrobenzene, CAS Reg. No. 610-39-9)
),5-Dinitrotoluene (1-Methyl-3,5-dinitrobenzene, CAS Reg. No. 618-85-9)
-Amino-4,6-dinitrotoluene (2-Methyl-3,5-dinitrobenzenamine, CAS Reg. No. 35572-78-2)
-Amino-2,6-dinitrotoluene (4-Methyl-3,5-dinitrobenzenamine, CAS Reg. No. 19406-51-0)
etranitromethane (CAS Reg. No. 509-14-8)
rinitroglycerin (1,2,3-Propanetriol trinitrate, CAS Reg. No. 55-63-0)
,2-Dinitroglycerin (1,2,3-Propanetriol 1,2-dinitrate, CAS Reg. No. 621-65-8)
,3-Dinitroglycerin (1,2,3-Propanetriol 1,3-dinitrate, CAS Reg. No. 623-87-0)
-Mononitroglycerin (1,2,3-Propanetriol 1-nitrate, CAS Reg. No. 624-43-1)
-Mononitroglycerin (1,2,3-Propanetriol 2-nitrate, CAS Reg. No. 620-12-2)
itrocellulose (Cellulose nitrate, CAS Reg. No. 9004-70-0)
hite Phosphorus (CAS Reg. No. 12185-10-3)
cute Toxicity
isposition
etabolism
mes test

20 (concluded)

,5-DNT and 4-ADNT were absorbed from the gastrointestinal tract, metabolized nd excreted in the urine.
n the Ames test, 1,3-dinitroglycerin (1,3-DNG), 1-mononitroglycerin (1-MNG), itrocellulose and white phosphorus were not mutagenic. Trinitrotoluene (TNT) ,4-DNT, 2,5-DNT, tetranitromethane (TNM) and 1,2-DNG were mutagenic at 10 to 0 µg/plate in one or more strains. TNM was bactericidal without activation. ,2-DNG was nonmutagenic with activation. 2,3-DNT, 2,6-DNT, 3,5-DNT, trinitro-lycerin and 2-MNG were weakly mutagenic, with mutagenic results at 100 or ,000 µg/plate in one or more strains.

PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under U.S. Department of the Army, Contract No. DAMD-17-74-C-4073, MRI Project No. 3900-B, "Munitions Compounds Mammalian Toxicity Study." The work was supported by the Medical Research and Development Command, Department of the Army. Captain John P. Glennon and Dr. Jack C. Dacre, Environmental Protection Research Division, USAMBRDL, were the successive contract officer's technical representatives for the project.

This work was conducted in the Biological Sciences Division under the direction of Dr. William B. House, between February 1, 1975 and February 28, 1978. The experimental work was directed by Dr. Cheng-Chun Lee, Principal Advisor for Pharmacology/Toxicology, with the assistance of Dr. Harry V. Ellis, III, Senior Pharmacologist. Dr. John R. Hodgson, Head, Biochemical and Developmental Pharmacology, supervised the studies on disposition and metabolism, assisted by Dr. Shang W. Hwang and Miss Laurel M. Halpap, and the Ames tests, assisted by Mr. Daniel L. VanGeethem. Dr. Danny O. Helton, Senior Chemist, prepared the 3,5-dinitrotoluene. Mr. Bruce S. Andersen assisted with the in vivo toxicity studies.

Approved for:

MIDWEST RESEARCH INSTITUTE

Cheng-Chun Lee

Cheng-Chun Lee
Principal Advisor for
Pharmacology/Toxicology

December 8, 1978

Accession For
NTIS GRA&I
DDC TAB
Unannounced
Justification
By
Distribution/
Availability Codes
Dist | Avail and/or special
A

TABLE OF CONTENTS

REPRODUCED FROM BEST AVAILABLE COPY

TABLE OF CONTENTS (continued)

List of Figures

List of Tables

List of Tables (concluded)

I. INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, entitled "Munitions Compounds Mammalian Toxicity Study," we have completed various studies in Phase I. Most results were reported in Progress Report No. 1, dated July 22, 1975.[1] Because sufficient compound was not yet available, several compounds were not tested until after that report was completed. This report includes the acute oral toxicity in rats and mice, primary skin and eye irritation in rabbits and dermal sensitivity in guinea pigs of 3,5-dinitrotoluene (3,5-DNT), 2-amino-4,6-dinitrotoluene (2-ADNT), and 4-amino-2,6-dinitrotoluene (4-ADNT), and the disposition and metabolism of ^{14}C-labeled 3,5-DNT and 4-ADNT after oral administration to rats.

The so-called "Ames Test"[2a,2b] is becoming more popular as a rapid screen for mutagenicity. Therefore, we have performed the test on available compounds, including tetranitromethane (TNM), 2,4,6-trinitrotoluene (TNT), 2,3-dinitrotoluene (2,3-DNT), 2,4-dinitrotoluene (2,4-DNT), 2,5-dinitrotoluene (2,5-DNT), 2,6-dinitrotoluene (2,6-DNT), 3,4-dinitrotoluene (3,4-DNT), 3,5-DNT, trinitroglycerin (TNG), 1,2-dinitroglycerin (1,2-DNG), 1,3-dinitroglycerin (1,3-DNG), 1-mononitroglycerin (1-MNG), 2-mononitroglycerin (2-MNG), nitrocellulose (NC), and white phosphorus (WP).

A corrigenda for Report No. 1 is also included.

Results of studies on the absorption and dispositon of ^{14}C-labeled NC were included in Report No. 5.[3] Further chemical analysis of NC fines was reported separately.[4]

II. MATERIALS AND METHODS

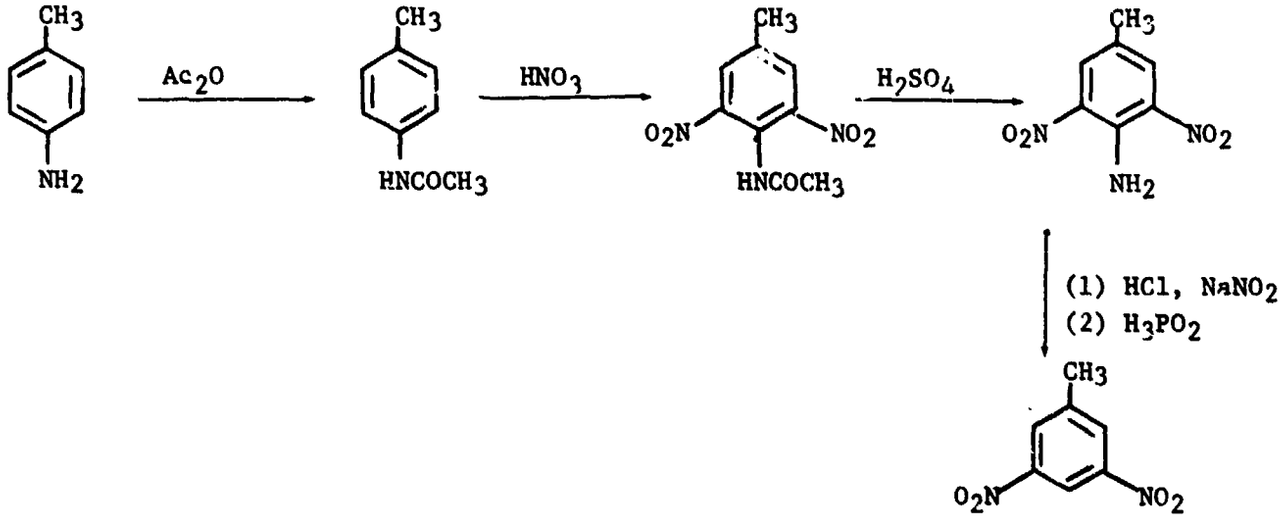
A. Animals

Male and female Charles River CD® rats were obtained from the Charles River Breeding Laboratories (North Wilmington, Massachusetts). Male and female albino Swiss mice were obtained from the National Laboratory Animal Company (O'Fallon, Missouri). Guinea pigs and New Zealand rabbits were obtained from Small Stock Industries (Pea Ridge, Arkansas).

All animals were kept in air-conditioned rooms (75 ± 5°F) with relative humidity of 50 ± 10% and photoperiod of 12 hr. They were kept under observation for 1 week after arrival; only healthy animals were used. Rats, mice, and guinea pigs were housed in plastic cages and provided with hardwood bedding; rabbits were housed in metal cages with wire bottoms.

B. Chemicals

3,5-DNT was prepared at Midwest Research Institute (MRI) by Dr. Danny O. Helton, according to the method of Morton and McGookin,[5] as outlined below. 2-ADNT and 4-ADNT were obtained from the late Dr. Lloyd A. Kaplan of the Naval Surface Weapons Center, White Oak (Silver Spring, Maryland). TNM was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. All other chemicals were those used earlier.[1]



C. Acute LD_{50}

Rats and mice were fasted for at least 16 hr prior to oral dosing. Oral doses of 5% suspensions in peanut oil were administered to the rodents by intragastirc iatubation via a stainless steel tube. After treatment, the survivors were observed daily for 14 days for delayed mortality or toxic signs. The LD_{50} was calculated for each compound by a computer program based on the maximum likelihood method of Finney.[6]

D. Primary Skin and Eye Irritation

Primary skin and eye irritation tests were carried out according to the modified Draize method as described in the Code of Federal Regulations.[7] Rabbits were clipped free of hair over the appropriate skin areas at least 24 hr prior to testing. After application of the test material, as a 50% paste with peanut oil, the irritation score on intact and abraded skin and the eye was evaluated at 24 and 72 hr.

E. Dermal Sensitivity Studies

Dermal sensitivity test was performed in guinea pigs according to the "maximization test" described by Magnusson and Kligman.[8] Test animals were clipped free of hair at least 24 hr prior to testing or prior to the final challenge with the test substances. The preparations of the nitrotoluenes used for the dermal sensitization tests were the same as those used for acute oral toxicity study.

F. Disposition and Metabolism

1. Radiochemicals

The ^{14}C-labeled 3,5-DNT and 4-ADNT (ring-UL-^{14}CO were prepared by the New England Nuclear Corporation (Boston, Massachusetts). Their specific activities were 2.29 and 4.06 mCi/mM, respectively. They were radiopure by thin-layer chromatography.

2. Experimental Procedures

Charles River CD® female rats weighing between 175 and 250 g were used. Each rat was fasted overnight before being given a single oral dose of approximately 1/10 of the LD_{50} of the test compound, spiked with 10 µCi of the ^{14}C-labeled compound. The test material was suspended in peanut oil and given via an intragastric tube at a volume of 1 ml/100 g body weight. After dosing, each rat was placed immediately in a "Roth-Delmar" metabolism cage[9] with food and water ad libitum. The chamber was

vented continuously with CO_2-free air at a rate of 150 ml/min. Expired CO_2 was collected by bubbling the air through six absorption columns connected in series. Each column contained 100 ml of 5% NaOH. Feces and urine were collected separately in the apparatus. At the termination of each experiment, the rat was anesthetized with ether and aortic blood collected in a heparinized syringe. Liver, kidneys, brain, lungs, and thigh muscle were removed, weighed, and representative samples taken for analysis of radioactivity. The gastrointestinal tract plus contents (GI) was removed and weighed. The GI and the feces were homogenized in three volumes of dioxane[10] and filtered; samples of the filtrate and the filtered residue were assayed for radioactivity.

3. Radioactive Assays

Aliquots of whole blood, tissue samples, and filtrate residues were digested in 2N NaOH. The carcasses were digested in 10 volumes of 6N NaOH. Blood samples were decolorized by dropwise addition of hydrogen peroxide. Samples of tissue digests were neutralized with Beckman BBS-2, solubilized in Beckman BBS-3, and counted in a toluene-PPO-dimethyl POPOP cocktail using a Packard Tricarb 3375 liquid scintillation spectrometer. Samples of plasma, urine, dioxane filtrates, and test radiochemicals were solubilized directly in BBS-3 and counted. $^{14}CO_2$ samples from the air traps were spotted on filter paper, dried, and counted. All data were corrected for background and quenching.

4. Thin-Layer Chromatography (TLC)

Precoated Silica Gel 60 plates (without fluorescent indicator, 0.25 mm thickness, E. M. Laboratories, Inc., Elmsford, New York) were used for all experiments. All samples were spotted 2.0 cm from the bottom of the plate and developed for a minimum of 10 cm. The solvents used were: (a) n-butanol:methanol:water (120:33:57, v/v/v); (b) petroleum ether: ethyl acetate (15:85); and (c) 1,2-dichloroethane:petroleum ether (25:75). Nitrotoluenes were detected using 5% diphenylamine spray reagent[11] followed by UV-irradiation.

G. Ames Test

1. Preparation of Test Compounds

All nitrotoluenes, nitroglycerins, and TNM were dissolved in dimethylsulfoxide at concentrations varying from 1 to 10 mg/ml. WP was prepared from a saturated solution in distilled water ("phossy water"). NC was prepared as a suspension of 1 to 5 mg/ml in distilled water.

2. Bacteria and Culture Media

The bacteria used in our experiments were the Salmonella typhimurium tester strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100. These tester strains are histidine auxotrophs and are used to detect base-pair substitutions (TA-100 and TA-1535) and frame-shift reverse mutations (TA-98, TA-1537, and TA-1538). All tester strains were obtained from Dr. Bruce Ames, University of California, Berkeley.

The selective medium for the histidine auxotrophs in the mutagenesis assays was Minimal Agar-Davis (Difco, Detroit, Michigan). Difco nutrient broth was used to prepare stock cultures.

3. Preparation of Rat Liver S-9 Fraction

The S-9 fraction from rat liver was prepared according to the procedure of Ames et al.[2a] Briefly, male Charles River CD® rats weighing 180 to 200 g were given intraperitoneal injections of sodium phenobarbital at a dose of 80 gm/kg of body weight for four consecutive days. Twenty-four hours after the last dose the rats were killed. The livers were aseptically removed, washed once in 10.0 ml of ice-cold, sterile 0.15 M KCl, put into 3.0 ml of ice-cold, sterile 0.15 M KCl/gm of liver. The liver was then minced with a sterile scalpel and scissors, transferred into a cold Teflon in glass homogenizer and homogenized (25% w/v) at low speed (three to five strokes). The homogenates were transferred into sterile centrifuge tubes and centrifuged for 10 min at 9,000 x g. The supernatant (S-9 fraction) was decanted; 2-ml aliquots were frozen in an ethanol-dry ice bath and stored at -80°C in a Revco freezer.

Prior to use, each batch of S-9 fraction was tested for its efficiency for metabolic activation. For these tests, the TA-100 and the TA-1535 strains were used with 7,12-dimethylbenzanthracene (20 µg/plate) and cyclophosphamide (200 µg/plate) as the reference mutagens, respectively. Increasing amounts of S-9 fraction were added to complete the test system and the cultures incubated as in the normal assay. Each assay was run against a batch of S-9 fraction previously determined to give satisfactory results with the test chemicals. The concentration of the S-9 fraction from the new batch was then adjusted to give results comparable to the reference batch.

4. Microsomal Activation System

The microsomal activation system used for the mutagenic assay contained per milliliter: S-9 fraction (0.08 ml), NADP (4 µM), glucose-6-phosphate (5 µM), KCl (33 µM), $MgCl_2$ (8 µM), and sodium phosphate buffer (100 µM), pH 7.4. The microsomal activation system was prepared fresh daily.

5. Assay Procedure

The histidine-requiring Salmonella typhimurium tester strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 were exposed to selected concentrations of each test compound. The standard concentrations were 1,000, 300, 100, and 10 µg/plate (0.1 ml) to insure a wide dosage range. For those compounds showing bactericidal activity, as indicated by a reduction in the number of spontaneous revertants in the treated plates relative to the control, additional concentrations of 1.0 to 0.01 µg/plate were used. For NC, the highest concentration used was 5,000 µg/plate. For WP, a saturated aqueous solution was then serially diluted to 1/2, 1/10, 1/33, and 1/100. All compounds were prepared for testing as described above and run in duplicate with and without metabolic activation. 7,12-Dimethylbenzanthracene (20 µg), benzo[a]pyrene (5 µg), and cyclophosphamide (200 µg) were used as positive controls to assure that all strains were capable of mutation during a particular experiment, and that the metabolic activation system was working properly. All cultures were incubated for 48 hr at 37°C and the number of revertant colonies on each plate counted. The mutagen assay was scored as the ratio of the number of colonies (total revertants) in the experimental plates over the number of colonies in the control plates (spontaneous revertants). This was taken as the mutagenic ratio. A compound was considered to be mutagenic if its mutagenic ration was $\geq$ 2.0.[2a/] Our criterion for a strong mutagen was a compound having a mutagenic ratio $\geq$ 2.0 at concentrations less than 100 µg/plate. A weak mutagen requires greater than 100 µg/plate to produce a mutagenic ratio $\geq$ 2.0.

III. RESULTS

A. Synthesis of 3,5-DNT

1. Identity

a. Capillary Melting Point

Observed - 91 to 92°C
Reported[5] - 93°C

b. Infrared Spectrum

The infrared (IR) spectrum (KBr wafer) of prepared 3,5-DNT was consistent with that reported.[12]

c. Nuclear Magnetic Resonance

The NMR spectrum in deuterated chloroform of prepared 3,5-DNT was consistent with that reported.[13]

2. Assay

a. Gas Chromatography (GC)

The prepared 3,5-DNT was studied using the following system:

Instrument: Varian 200
Detector: Flame ionization
Column: 6 ft x 18/ in., glass
1.5% GEXE-60
1.5% DC-LSX-3-0295 on Gas Chrom Q
Column temperature: 170°C
Injector temperature: 175°C
Detector temperature: 200°C
Nitrogen flow: 40 cc/min

By peak area comparison, the prepared sample contains 98% 3,5-DNT and 2% of an unknown component with a retention time of 1.7 relative to that of the 3,5-DNT.

PRECEDING PAGE BLANK

b. Thin-Layer Chromatography (TLC)

1. Plate: Brinkman Silica Gel F
2. Solvent system: Ethyl acetate:petroleum ether (15:85)
3. Material spotted: 100, 10, 5, 2, and 1 µg sample
4. Detection: UV, 254 nm

The prepared sample showed a major spot at R_f 0.69 and a minor spot at R_f 0.77. Based on spot intensity, the minor spot has a concentration of 2%.

c. Discussion

TLC and GC results indicate the sample to be 98% pure with 2% of an organic impurity. Based on the synthesis method and sample workup procedures, the impurity may be an isomeric nitrotoluene.

B. In Vivo Studies

The acute oral LD_{50}s of all nitrotoluene derivatives tested, including those previously reported,[1/] in rats and mice are listed in Tables 1 and 2, respectively. The results of the primary skin and eye irritation tests in rabbits are shown in Tables 3 and 4. The results of the dermal sensitivity tests in guinea pigs are listed in Table 5.

1. 3,5-Dinitrotoluene

The acute $LD_{50} \pm$ S.E. (95% confidence limits) of 3,5-DNT in male and female rats were 309 ± 13 (278-340) and 216 ± 19 (160-256) mg/kg, respectively (Table 1). In male and female mice, they were 611 ± 43 (523-714) and 607 ± 21 (559-650) mg/kg, respectively (Table 2). No toxic signs except ataxia of some animals given the higher doses were noted.

3,5-DNT was non-irritating to rabbit skin and eyes and was not a sensitizing agent to guinea pigs (Tables 3, 4 and 5).

Table 6 summarizes the recovery of radioactivity after a single oral dose of 3,5-DNT (ring-UL-^{14}C). The radioactivity remaining in the GI tract averaged 65.0% and 2.9% of the administered dose at the end of 4 and 24 hr, respectively. The recovery of radioactivity in the feces during the same time periods were 0.9% and 21.7%. From these values, we estimate that at least 75% of the dose was absorbed and that the absorption was essentially complete at 24 hr. Most of the absorbed radioactivity was excreted in the urine, averaging 77.2% of the administered dose at 24 hr. No radioactivity was recovered in the expired air. Only small amounts of radioactivity were found in the various tissues. At 4 hr, the radioactivity ratios in the

tissues, relative to plasma, were in the following order: kidneys > liver > spleen, lungs > muscle, brain. At 24 hr, the radioactivity ratios of liver and muscle were approximately twice that at 4 hr, whereas the radioactivity ratios of the other tissues remained about the same.

The TLC analysis of 24-hr urine from 3,5-DNG (ring-UL-^{14}C)-treated rats using ethyl acetate:petroleum ether and butanol:methanol:water solvent systems demonstrated a single radioactive band which did not correspond to the parent compound (Figure 1). This radioactive component(s) had an R_f value of 0.65 with the butanol:methanol:water system.

2. 2-Amino-4,6-Dinitrotoluene

The acute $LD_{50} \pm$ S.E. (95% confidence limits) of 2-ADNT in male and female rats were 2,240 ± 85 (2,070-2,430) and 1,394 ± 191 (989-2,830) mg/kg, respectively (Table 1). In male and female mice, they were 1,722 ± 154 (1,450-2,131) and 1,522 ± 71 (1,372-1,692) mg/kg, respectively (Table 2). In both species, the toxic signs were inactivity, depression, unconsciousness and slow, gasping breathing. In lethal doses, symptoms were obvious within 30 min, and most deaths occurred 1 to 24 hr after dosing. Although 2-ADNT is bright yellow, the surviving rats and mice excreted orange urine for a few days after dosing. Rats, but not mice, who survived the initial depression were hyperactive for several days. Their gait was hopping; they appeared on the verge of convulsing, although when disturbed they would jump several inches in the air rather than convulse. They appeared extremely unkempt, with urine-stained, bristly hair. Most of these rats returned to normal in 7 to 10 days, but some rats and some mice died as long as 10 days after dosing.

2-ADNT was a mild skin irritant (Table 3), but non-irritating to the rabbits' eyes (Table 4). It was not a sensitizing agent in guinea pigs (Table 5).

3. 4-Amino-2,6-Dinitrotoluene

The acute $LD_{50} \pm$ S.E. (95% confidence limits) of 4-ADNT in male and female rats were 1,360 ± 53 (1,260-1,465) and 959 ± 76 (787-1,154) mg/kg, respectively (Table 1). In male and female mice, they were 1,342 ± 107 (1,141-1,611) and 1,495 ± 90 (1,318-1,713) mg/kg, respectively. Toxic signs included inactivity, depression, unconsciousness, and slow, gasping breathing, and appeared within 30 min of dosing. Most deaths occurred 1 to 24 hr after dosing. However, some animals recovered from the depression within a day, then died as long as 10 days after dosing. Mice dosed with the orange 4-ADNT excreted reddish-orange urine, which stained their hair for a few days. Rats recovering from the initial depression were hyperactive, with a hopping gait and extremely unkempt appearance, for several days.

4-ADNT was not irritating to rabbits' skins and eyes and was not a sensitizing agent in the guinea pigs (Tables 3, 4 and 5).

Table 7 summarizes the recovery of radioactivity after a single oral dose of 4-ADNT (ring-UL-^{14}C). The radioactivity remaining in the GI tract averaged 70.1% and 5.5% of the administered dose at the end of 4 and 24 hr, respectively. The recovery of radioactivity in the feces during these same time periods were 0.6% adn 44.4%. From these values, we estimate that at least 50% of the dose was absorbed and that the absorption was essentially complete at 24 hr. Most of the absorbed radioactivity was excreted in the urine, averaging 30.1% of the administered dose at 24 hr. A negligible amount (0.2%) of the radioactivity was recovered in the expired air. Only small amounts of radioactivity were found in the various tissues. At 4 hr, the radioactivity ratios in the tissues, relative to plasma, were in the following order: kidneys > liver > spleen > lungs > brain > muscle. At 24 hr, the radioactivity ratio of the liver was three times that at 4 hr; the ratios of kidney and lung doubled, whereas the radioactivity ratios of the other tissues remained about the same.

TLC analysis of 24-hr urine from 4-ADNT (ring-UL-^{14}C)-treated rats using ethyl acetate:petroleum ether and butanol:methanol:water solvent systems demonstrated two radioactive components, neither of which corresponded to the parent compound (Figure 2). Both components migrated with the butanol:methanol:water solvent system. The first, more polar component had an R_f value of 0.57 and represented about 78% of the total radioactivity in the urine. The other component had an R_f value of 0.87 and represented 22% of the total radioactivity in the urine.

C. Ames Tests

The mutagenic activity of the munitions compounds tested is presented in Table 8 as mutagenic ratios. Table 9 summarizes the critical values. Four compounds including 1,3-DNG, 1-MNG, NC and WP were not mutagenic in the test system. The other 11 compounds displayed various degrees of mutagenic activity.

Five compounds including TNT, 2,4-DNT, 2,5-DNT, TNM, and 1,2-DNG, exhibited significant increases in the number of revertants at 10 or 30 µg/plate. With the exception of 1,2-DNG, these compounds produced both base-pair substitution and frame-shift mutations. 1,2-DNG produced only frame-shift mutation as indicated by the reversion of the TA-1537 strain. TNT appeared to be the most potent of the compounds tested. As little as 10 µg/plate of TNT was mutagenic in the TA-98, TA-1538 and TA-1537 strains.

When 30 µg/plate were used, TNT was positive in four test strains; at 300 µg/plate, TNT was positive in all five tester strains. 2,5-DNT showed positive response in all five tester strains but at a higher concentration than TNT. TNM was bactericidal at concentrations above 1.0 µg/plate without activation; however, S-9 fraction reduced the toxicity and produced a mutagenic response in strains TA-1535 and TA-98. On the other hand, the mutagenic activity of 2,4-DNT was decreased and that of 1,2-DNG was completely inactivated in the presence of S-9 microsomal fraction. 1,2-DNG was mutagenic in the TA-1537 strain without activation.

Six compounds including 2,3-DNT, 2,6-DNT, 3,4-DNT, 3,5-DNT, TNG, and 2-MNG displayed a weak mutagenic activity in these test systems. These compounds required between 100 and 1,000 µg/plate to produce a significant increase in mutation frequency with or without metabolic activation. 3,4-DNT and 2-MNG produced only base-pair substitution mutation; 2,3-DNT and 2,6-DNT produced only frame-shift mutations; 3,5-DNT and TNG produced both base-pair substitution and frame-shift mutations. 2,3-DNT and 2-MNG required metabolic activation. On the other hand, the mutagenic activity of 3,5-DNT was decreased and that of 2,6-DNT was completely inactivated in the presence of the S-9 microsomal fraction.

TABLE 1

AUTE ORAL TOXICITIES (mg/kg) OF VARIOUS NITROTOLUENE COMPOUNDS IN MALE AND FEMALE RATS

Compounds	Males			Females		
	$LD_{50} \pm$ S.E.	95% Confidence Limits	Slope $\pm$ S.E.	$LD_{50} \pm$ S.E.	95% Confidence Limits	Slope $\pm$ S.E.
TNT	1,010 $\pm$ 41	922 - 1,108	5.96 $\pm$ 1.50	820 $\pm$ 32	747 - 889	5.68 $\pm$ 1.41
2,3-DNT	1,102 $\pm$ 20	1,011 - 1,169	15.46 $\pm$ 6.84	911 $\pm$ 65	584 - 1,049	3.25 $\pm$ 1.37
2,4-DNT	568 $\pm$ 59	434 - 705	2.25 $\pm$ 0.61	650 $\pm$ 49	520 - 743	3.53 $\pm$ 0.94
2,5-DNT	616 $\pm$ 34	532 - 707	4.20 $\pm$ 1.37	517 $\pm$ 25	477 - 575	5.56 $\pm$ 1.85
2,6-DNT	535 $\pm$ 58	397 - 646	1.79 $\pm$ 0.41	795 $\pm$ 22	744 - 844	8.48 $\pm$ 2.14
3,4-DNT	907 $\pm$ 42	815 - 1,011	4.78 $\pm$ 1.28	807 $\pm$ 33	721 - 874	4.67 $\pm$ 1.23
3,5-DNT	309 $\pm$ 13	278 - 340	5.03 $\pm$ 1.48	216 $\pm$ 19	160 - 256	3.44 $\pm$ 1.11
2-ADNT	2,240 $\pm$ 85	2,070 - 2,430	8.97 $\pm$ 3.41	1,394 $\pm$ 191	989 - 2,830	1.85 $\pm$ 0.79
4-ADNT	1,360 $\pm$ 53	1,260 - 1,465	18.17 $\pm$ 2.34	959 $\pm$ 76	787 - 1,154	3.60 $\pm$ 1.07

TABLE 2

ACUTE ORAL TOXICITIES (mg/kg) OF VARIOUS NITROTOLUENE COMPOUNDS IN MALE AND FEMALE MICE

Compounds	Males			Females		
	$LD_{50} \pm$ S.E.	95% Confidence Limits	Slope $\pm$ S.E.	$LD_{50} \pm$ S.E.	95% Confidence Limits	Slope $\pm$ S.E.
TNT	1,014 $\pm$ 52	905 - 1,163	3.47 $\pm$ 1.06	1,009 $\pm$ 54	880 - 1,117	3.86 $\pm$ 0.88
2,3-DNT	1,372 $\pm$ 34	1,285 - 1,441	8.53 $\pm$ 2.26	1,089 $\pm$ 32	1,029 - 1,175	7.96 $\pm$ 2.03
2,4-DNT	1,954 $\pm$ 68	1,848 - 2,178	4.50 $\pm$ 1.15	1,340 $\pm$ 67	1,205 - 1,500	4.15 $\pm$ 0.93
2,5-DNT	652 $\pm$ 28	585 - 712	5.05 $\pm$ 1.29	659 $\pm$ 12	633 - 690	12.97 $\pm$ 3.51
2,6-DNT	621 $\pm$ 51	488 - 721	3.25 $\pm$ 0.87	807 $\pm$ 35	725 - 893	5.93 $\pm$ 1.51
3,4-DNT	859 $\pm$ 37	787 - 958	4.19 $\pm$ 1.10	747 $\pm$ 26	702 - 821	5.74 $\pm$ 1.28
3,5-DNT	611 $\pm$ 43	523 - 714	2.90 $\pm$ 0.70	607 $\pm$ 21	559 - 650	5.95 $\pm$ 1.45
2-ADNT	1,722 $\pm$ 154	1,450 - 2,131	2.00 $\pm$ 0.42	1,522 $\pm$ 71	1,372 - 1,692	5.93 $\pm$ 1.50
4-ADNT	1,342 $\pm$ 107	1,141 - 1,611	2.32 $\pm$ 0.49	1,495 $\pm$ 90	1,318 - 1,713	3.69 $\pm$ 0.81

TABLE 3

PRIMARY SKIN IRRITATION OF VARIOUS NITROTOLUENE COMPOUNDS IN RABBITS

Compounds	Primary Irritation Score[a/]
TNT	1.0[b/]
2,3-DNT	1.78
2,4-DNT	0.25[c/]
2,5-DNT	3.80
2,6-DNT	0.21[d/]
3,4-DNT	2.00
3,5-DNT	< 0.2
2-ADNT	0.21
4-ADNT	< 0.2
Peanut Oil (vehicle control)	0.33

a/ Average value of six rabbits with intact and abraded skin in each test group. The compounds are classified as follows:
> 0.2 over controls is mild irritant
> 2.5 over controls is moderate irritant
> 5.0 over controls is severe irritant

b/ Red color under all patches at 24 hours.

c/ No edema was apparent but the entire area covered by the compound was undergoing necrosis in 24 hours in both the intact and abraded skin.

d/ Yellow color under all patches at 24 hours.

TABLE 4

PRIMARY EYE IRRITATION OF VARIOUS NITROTOLUENE COMPOUNDS IN RABBITS

Compounds	Results[a]
TNT[b]	Nonirritant
2,3-DNT	Nonirritant
2,4-DNT	Nonirritant
2,5-DNT	Nonirritant
2,6-DNT	Nonirritant
3,4-DNT[c]	Nonirritant
3,5-DNT	Nonirritant
2-ADNT	Nonirritant
4-ADNT	Nonirritant
Peanut Oil (vehicle control)	Nonirritant

a/ Six rabbits per test group.
b/ Red color around the eye at 24 hours.
c/ Yellow color around the eye at 24 hours.

TABLE 5

DERMAL SENSITIVITY OF VARIOUS NITROTOLUENE COMPOUNDS IN GUINEA PIGS

Compound	Number Responding	% Response	Sensitisation
TNT	4/10	40%	moderate
2,3-DNT	0/10	0	none
2,4-DNT	0/10	0	none
2,5-DNT	0/10	0	none
2,6-DNT	2/10	20%	mild
3,4-DNT	0/10	0	none
3,5-DNT	0/10	0	none
2-ADNT	0/10	0	none
4-ADNT	0/10	0	none

TABLE 6

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING 3,5-DNT-(RING-UL-^{14}C)

	% of Administered Dose		Radioactivity Ratio[e]	
	4 Hour	24 Hour	4 Hour	24 Hour
Gastrointestinal tract plus contents	65.0 ± 6.3[c]	2.9 ± 1.0		
Feces	0.9 ± 0.3	21.7 ± 3.7		
Whole Blood[a]	1.1 ± 0.3	0.1 ± 0.0		
Expired Air	ND	0.0[d]		
Urine	24.6 ± 3.3	77.2 ± 2.2		
Spleen	0.1 ± 0.0	< 0.1	0.6 ± 0.1	0.6 ± 0.1
Liver	0.8 ± 0.2	0.1 ± 0.0	1.0 ± 0.2	2.5 ± 0.1
Kidneys	0.5 ± 0.1	< 0.1	2.6 ± 0.3	2.9 ± 0.4
Brain	0.1 ± 0.0	< 0.1	0.3 ± 0.0	0.4 ± 0.0
Lungs	0.1 ± 0.0	< 0.1	0.6 ± 0.0	0.6 ± 0.1
Skeletal Muscle[b]	2.9 ± 0.6	0.3 ± 0.0	0.3 ± 0.0	0.6 ± 0.1
Recovery	96.6 ± 2.7	103.8 ± 2.2		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of three rats.
d/ Mean of two rats.
e/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.
Note: ND = Not determined

TABLE 7

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING 4-AMINO-2,6-DINITROTOLUENE-(RING-UL-^{14}C)

	% of Administered Dose		Radioactivity Ratio	
	4 Hours	24 Hours	4 Hours	24 Hours
GI and contents	70.1 ± 4.1[c/]	5.5 ± 1.1		
Feces	0.6 ± 0.1	44.4 ± 12.4		
Whole blood[a/]	0.6 ± 0.2	0.2 ± 0.0		
Air	ND	0.2 ± 0.1		
Urine	11.1 ± 3.5	30.1 ± 9.5		
Spleen	< .1	< .1	1.4 ± 0.6	1.2 ± 0.1
Liver	0.9 ± 0.2	0.5 ± 0.1	2.6 ± 0.5	8.2 ± 0.6
Kidneys	0.4 ± 0.1	0.1 ± 0.0	4.8 ± 0.7	7.7 ± 0.4
Brain	0.1 ± 0.0	< 0.1	0.9 ± 0.1	0.8 ± 0.2
Lungs	0.1 ± 0.0	< 0.1	1.3 ± 0.2	2.0 ± 0.2
Muscle[b/]	2.4 ± 0.6	0.3 ± 0.0	0.6 ± 0.1	0.6 ± 0.1
Recovery	86.4 ± 8.1	81.2 ± 4.5		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean + S.E. of three rats.
d/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.
Note: ND = Not determined.

TABLE 8

MUTAGENICITY OF MUNITIONS COMPOUNDS USING THE SALMONELLA/MICROSOME PLATE TEST

Test Compound	µg[b]	Mutagenic Ratio[a] TA-100 I[c]	TA-100 II[d]	TA-1535 I	TA-1535 II	TA-98 I	TA-98 II	TA-1537 I	TA-1537 II	TA-1538 I	TA-1538 II
TNT	10	1.5	1.5	1.0	1.1	14.8*	5.6*	4.3*	2.0*	26.0*	2.3*
	30	2.6*	2.3*	0.9	1.4	17.8*	18.2*	5.2*	4.0*	44.8*	8.8*
	100	4.6*	2.6*	1.3	1.3	7.9*	4.8*	6.5*	3.2*	34.5*	21.9*
	300	-	7.9*	0.9	2.4*	11.3*	7.2*	-	9.0*	17.8*	13.2*
	1,000	-[e]	-	-	-	-	12.5*	-	-	-	11.5*
2,3-DNT	30	1.0	1.0	0.8	0.8	0.8	1.2	0.7	1.3	1.1	1.3
	100	0.9	1.4	0.6	0.6	1.0	1.5	1.0	1.0	0.9	1.0
	300	1.6	1.0	0.5	1.0	1.1	0.7	1.2	0.9	1.9	1.2
	1,000	-	-	-	0.9	-	1.0	0.3	1.8	0.1	2.8*
2,4-DNT	10	0.8	0.9	1.4	1.2	1.0	0.9	1.2	0.8	2.0*	0.8
	30	0.9	1.2	1.0	0.9	1.0	1.1	0.9	0.8	3.0*	1.8
	100	1.0	1.1	0.8	0.6	1.5	1.8	0.9	1.2	6.6*	1.3
	300	2.0*	2.2*	1.1	1.3	1.2	1.8	1.6	1.4	13.5*	1.2
	1,000	2.8*	4.2*	0.5	1.6	0.8	1.3	-	1.8	-	4.8*
2,5-DNT	30	1.1	1.2	1.5	1.0	1.6	1.6	0.6	1.7	3.6*	2.3*
	100	1.4	1.3	0.8	1.6	2.0*	2.2*	2.6*	2.8*	13.2*	5.5*
	300	2.1*	2.5*	-	1.8	2.8*	3.4*	1.0	2.4*	7.8*	14.3*
	600	-	5.2*	-	3.7*	-	2.2*	2.8*	2.8*	-	17.5*
	1,000	-	5.7*	-	-	-	1.9	-	-	-	-

TABLE 8 (continued)

Test Compound	µg[b]	Mutagenic Ratio[a] TA-100 I[c]	TA-100 II[d]	TA-1535 I	TA-1535 II	TA-98 I	TA-98 II	TA-1537 I	TA-1537 II	TA-1538 I	TA-1538 II
2,6-DNT	30	1.3	1.2	0.8	1.1	1.4	1.4	1.1	0.8	1.6	0.9
	100	1.2	1.1	0.9	0.8	1.2	1.1	1.3	1.0	1.4	1.0
	300	1.7	1.6	1.0	1.2	1.2	1.1	1.2	0.6	2.7*	0.5
	1,000	1.6	1.3	1.3	1.0	1.5	0.7	0.6	1.8	4.9*	1.0
3,4-DNT	10	1.1	0.9	1.4	1.0	1.0	1.0	0.9	0.5	0.8	0.9
	100	1.1	1.1	-	0.6	0.8	0.9	1.4	0.8	1.0	1.0
	500	1.4	1.6	-	0.7	1.0	0.6	0.6	0.8	1.8	1.0
	1,000	2.6*	4.6*	-	0.3	0.4	0.9	-	0.8	0.2	1.7
3,5-DNT	10	1.4	1.0	1.4	0.6	1.0	0.6	1.1	1.2	1.6	0.9
	100	1.1	0.9	1.5	0.7	1.2	1.1	1.8	1.2	5.0*	1.8
	500	3.0*	1.9	1.1	1.3	7.7*	3.4*	2.8*	2.8*	29.2*	14.4*
	1,000	2.4*	5.5*	-	1.1	4.7*	8.0*	-	5.9*	-	52.1*
TNM	0.01	0.9	ND[f]	0.9	ND	0.9	ND	0.9	ND	0.9	ND
	0.05	1.2	ND	0.7	ND	0.7	ND	1.1	ND	1.6	ND
	1.0	1.0	0.8	-	0.9	1.1	1.0	1.1	0.8	1.3	0.7
	10.0	-	1.0	-	1.0	-	0.8	-	0.8	-	0.7
	30.0	-	1.2	-	10.8*	0	3.4*	-	0.8	-	0.7
	100.0	-	1.4	-	8.4*	-	1.5	-	1.2	-	0.6
	300.0	-	-	-	4.0*	-	1.4	-	0.8	-	0.9
TNG	10	1.4	0.7	1.0	1.0	1.2	1.0	0.8	1.2	1.0	0.4
	100	0.9	0.7	0.9	1.0	1.0	0.9	0.9	0.8	1.0	0.8
	300	1.0	1.0	0.8	1.8	1.5	0.9	1.0	1.0	-	0.4
	1,000	0.8	0.7	0.8	4.0*	0.6	0.6	4.0*	0.5	-	0.2

TABLE 8 (concluded)

Test Compound	µg[b]	Mutagenic Ratio[a] TA-100 I[c]	TA-100 II[d]	TA-1535 I	TA-1535 II	TA-98 I	TA-98 II	TA-1537 I	TA-1537 II	TA-1538 I	TA-1538 II
1,3-DNG	10	1.4	0.6	1.0	0.8	1.3	0.6	1.0	1.0	0.8	0.9
	100	1.4	1.0	1.6	1.3	1.4	0.8	0.9	0.7	1.2	1.0
	300	1.0	1.2	1.7	1.3	1.5	0.9	0.7	1.0	1.3	0.8
	1,000	-	0.3	-	1.0	0.2	0.8	-	0.4	-	0.9
1,2-DNG	10	1.2	0.8	0.6	1.2	1.4	1.2	2.3*	0.7	0.7	0.8
	100	1.0	0.9	0.7	1.0	1.6	1.4	1.7	0.6	0.8	1.0
	300	1.1	1.0	0.8	1.0	1.0	1.2	2.0*	0.8	0.7	0.9
	1,000	1.2	1.0	0.9	0.9	1.4	1.4	2.0*	0.6	0.7	0.9
1-MNG	10	1.0	0.9	1.0	1.1	1.0	1.0	1.2	1.0	0.9	1.2
	100	0.9	1.2	1.5	1.0	0.7	1.4	1.2	1.2	0.6	1.2
	300	1.0	1.2	1.3	1.7	0.9	1.3	1.2	0.8	1.0	1.2
	1,000	0.8	1.1	1.0	0.7	1.4	1.0	1.5	0.8	0.7	1.9
2-MNG	10	1.3	1.0	1.5	1.2	1.0	0.9	0.6	1.5	1.2	0.8
	100	1.6	1.2	1.7	1.1	0.7	0.6	0.6	2.0	1.4	1.0
	300	1.5	1.0	1.3	1.8	1.0	0.9	0.9	2.0	1.3	0.9
	1,000	1.2	1.2	1.7	2.3*	0.7	0.8	1.0	0.8	1.4	0.9
NC	100	0.8	0.9	0.6	1.2	1.0	0.8	1.3	0.6	0.9	1.6
	1,000	0.9	1.0	1.0	0.8	1.1	0.9	1.2	0.6	1.0	1.1
	5,000	0.8	1.0	0.8	0.9	1.0	1.0	1.2	0.6	1.3	1.4
WP	100 µl	0.9	1.0	0.7	0.4	1.4	1.2	0.6	0.7	1.3	1.2

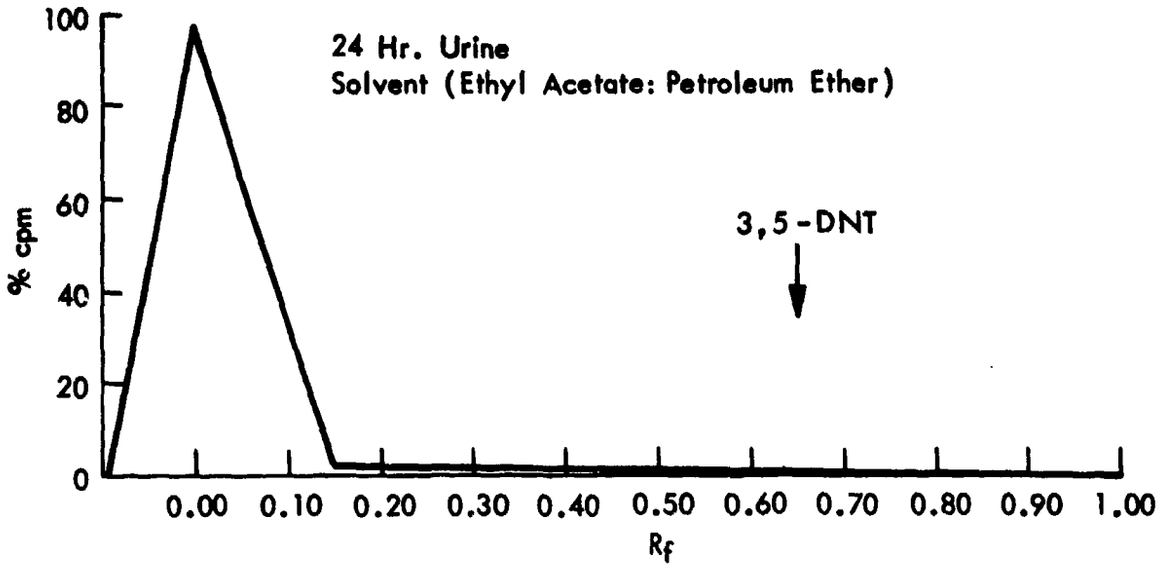
a/ Mutagenic Ratio: number of histidine revertants in the test culture/number of histidine revertants in the control dish.
b/ µg of test compound/plate introduced into 2.0 ml of top agar.
c/ Test run without metabolic activation.
d/ Test run with metabolic activation.
e/ Microbial toxicity.
f/ ND = not determined.
* Classified as mutagenic (M.R. ≥ 2,0).

TABLE 9

SUMMARY OF MUTAGENICITY OF MUNITIONS COMPOUNDS USING THE SALMONELLA/MICROSOME PLATE TEST

Compound	µg/Plate[a] ± Met Act[b]	Revertant Colonies/Plate TA-100	TA-1535	TA-98	TA-1537	TA-1538	Mutagenic[e] Level (µg) ± Met Act	Toxic Level (µg) ± Met Act
TNT	30-	419(160)[c,d]	38(44)	410(23)[d]	42(8)[d]	807(12)[d]	10-	300-
	30+	296(130)[d]	23(16)	382(21)[d]	40(10)[d]	350(20)[d]	10+	1,000+
2,3-DNT	1,000-	0(200)	0(48)	0(36)	4(13)	2(16)	NM-[f]	> 1,000-
	1,000+	0(150)	18(20)	27(26)	16(9)	56(20)[d]	1,000+	≥ 1,000+
2,4-DNT	1,000-	516(184)[d]	14(27)	34(42)	0(8)	0(8)	10-	≥ 1,000-
	1,000+	686(161)[d]	24(15)	38(30)	18(10)	76(16)[d]	30+	≥ 1,000+
2,5-DNT	300-	376(177)[d]	0(26)	96(34)[d]	7(7)	78(10)[d]	30-	600-
	600+	756(146)[d]	52(14)[d]	70(33)[d]	20(7)[d]	263(15)[d]	30+	≥ 1,000+
2,6-DNT	1,000-	281(179)	72(55)	22(15)	7(11)	88(18)[d]	300-	≥ 1,000-
	1,000+	164(124)	14(14)	19(26)	14(8)	23(22)	NM+	≥ 1,000+
3,4-DNT	1,000-	318(120)[d]	0(22)	18(40)	0(11)	3(18)	1,000-	≥ 1,000-
	1,000+	693(152)	6(20)	32(37)	6(8)	44(26)	1,000+	≥ 1,000+
3,5-DNT	1,000-	316(130)[d]	0(22)	156(33)[d]	0(11)	0(14)	100-	≥ 1,000-
	1,000+	756(120)[d]	25(23	272(34)[d]	47(8)[d]	1,252(24)[d]	500+	≥ 1,000+
TNM	1-	135(142)	32(44)	32(28)	8(7)	9(7)	NM-	10-
	30+	152(129)	140(13)[d]	76(22)[d]	10(12)	14(20)	30+	≥ 300+
TNG	1,000-	133(170)	36(42)	21(36)	40(10)[d]	0(3)	1,000-	≥ 1,000-
	1,000+	105(157)	49(18)[d]	18(32)	6(12)	2(15)	1,000+	≥ 1,000+
1,2-DNG	10-	240(208)	42(69)	33(23)	16(7)[d]	14(19)	10-	≥ 1,000-
	1,000+	250(238)	58(63)	29(20)	6(10)	20(22)	NM+	≥ 1,000+
1,3-DNG	300	185(176)	82(48)	30(20)	8(12)	13(10)	NM-	≥ 1,000-
	1,000+	79(248)	67(64)	26(32)	4(10)	18(20)	NM+	≥ 1,000+
1-MNG	1,000-	157(202)	16(15)	29(20)	12(8)	12(17)	NM-	> 1,000-
	1,000+	171(152)	10(15)	33(32)	6(8)	31(16)	NM+	> 1,000+
2-MNG	1,000-	163(133)	45(26)	22(33)	8(8)	16(11)	NM-	> 1,000-
	1,000+	166(143)	42(18)[d]	29*38)	10(12)	20(22)	1,000+	> 1,000+
NC	5,000-	186(241)	45(56)	44(42)	15(12)	16(12)	NM-	> 5,000-
	5,000+	202(204)	18(20)	38(36)	8(11)	20(14)	NM+	> 5,000+
WP	Undiluted	153(190)	26(37)	14(10)	8(13)	23(18)	NM-	NT
	Undiluted	152(160)	11(25)	20(16)	5(7)	24(20)	NM+	NT

a/ Concentration which caused mutations in the greatest number of tester strains or highest concentration tested.
b/ Without (-) and with (+) metabolic activation.
c/ Numbers enclosed by parentheses are the spontaneous reversion rates for each test.
d/ Classified as mutagenic, the mutagenic index (treated/control) is equal to or greater than 2.0.[2a/]
e/ Lowest concentration tested causing a two-fold increase in the mutation rate in at least one tester strain.
f/ NM = nonmutagenic at the highest concentration tested.



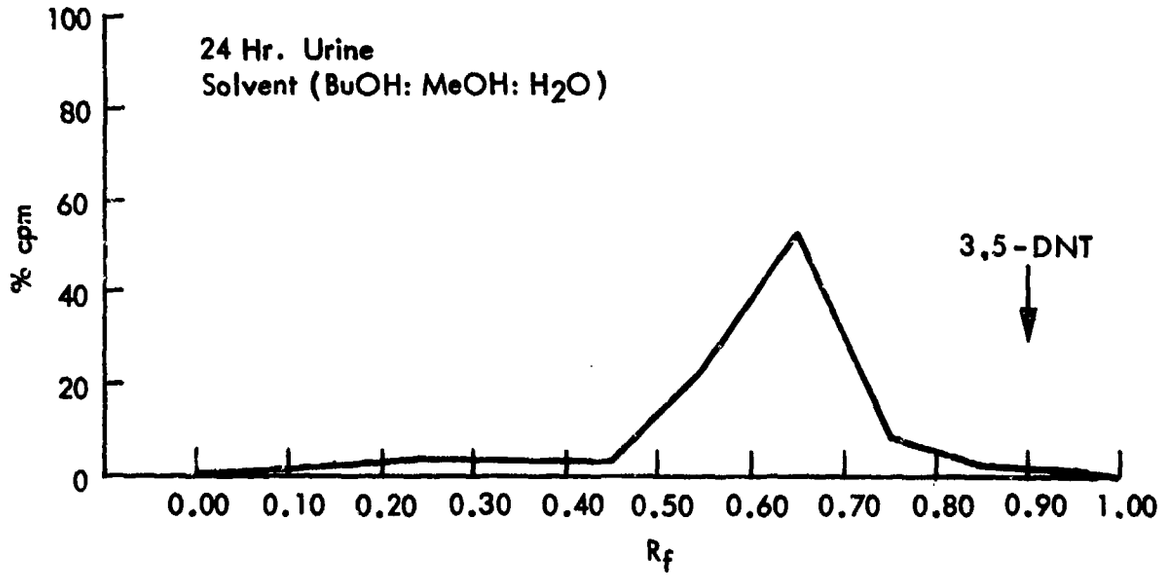
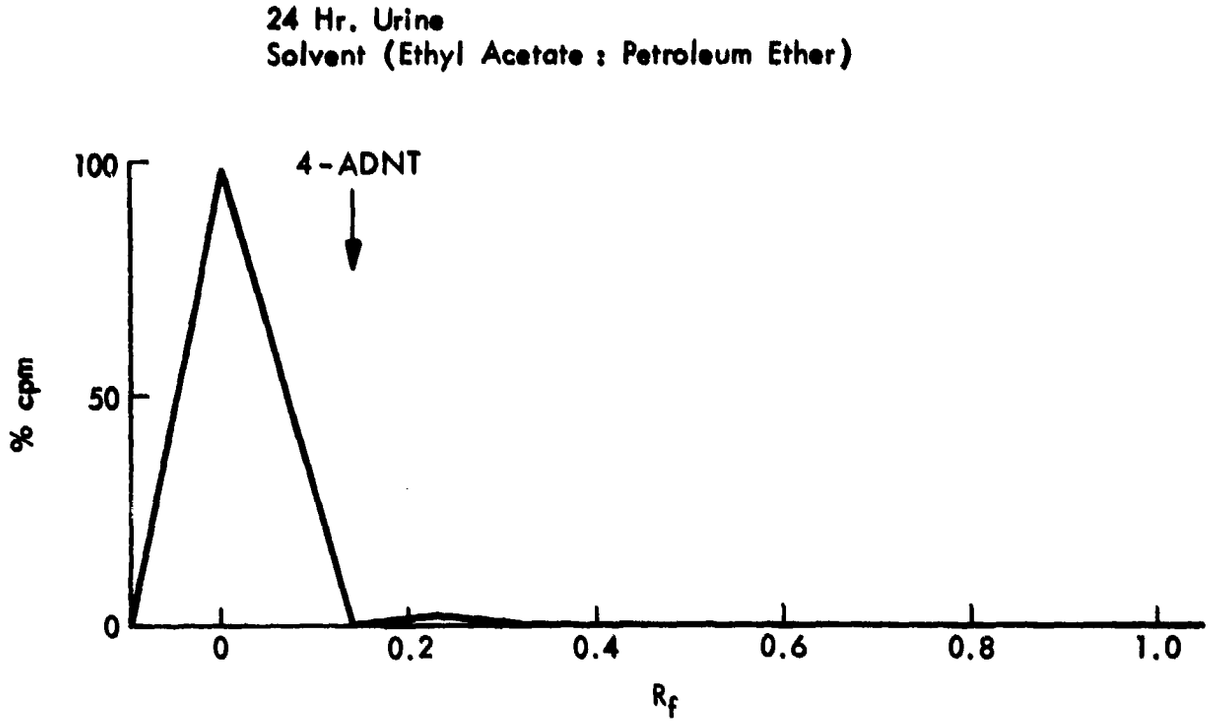


Figure 1 - TLC of Rat Urine After Oral Administration of 3,5-DNT (Ring-UL-^{14}C)



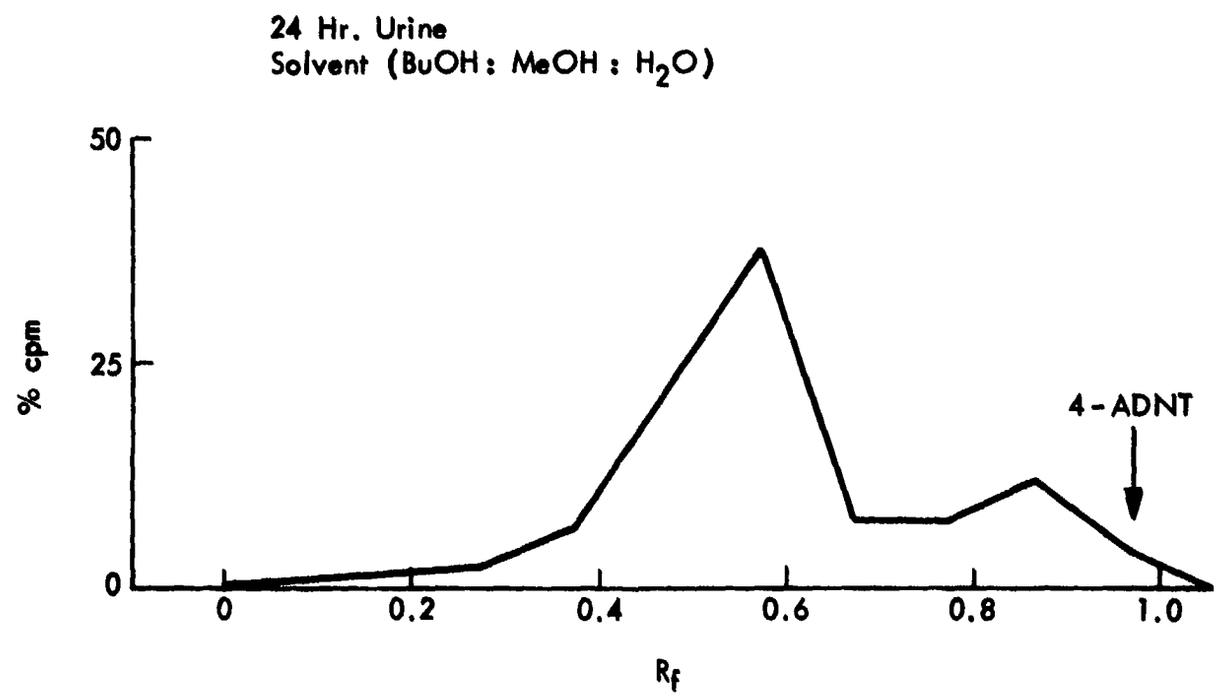


Figure 2 - TLC of Rat Urine After Oral Administration of 4-ADNT (Ring-UL-^{14}C)

IV. DISCUSSION

A. Acute Oral Toxicity in Rats

In male and female rats, 3,5-DNT was the most toxic nitrotoluene compound (LD_{50}s of 309 and 216 mg/kg, respectively), 2-ADNT was the least toxic nitrotoluene (LD_{50}s of 2,240 and 1,394 mg/kg, respectively) and 4-ADNT was the second least toxic nitrotoluene (LD_{50}s of 1,360 and 959 mg/kg, respectively). The two ADNTs were distinctly less toxic than TNT itself, while 2,3-DNT and 3,4-DNT, the two orthodinitro isomers, had toxicity similar to TNT. In the other nitrotoluenes, the two nitro groups are meta or para to each other, and toxicity was intermediate between that of TNT and that of 3,5-DNT.

B. Acute Oral Toxicity in Mice

As with the rats, 3,5-DNT was the most toxic nitrotoluene compound tested with LD_{50}s in male and female mice of 611 and 607 mg/kg, respectively. In male mice, 2-ADNT (LD_{50} of 1,722 mg/kg) and 4-ADNT (1,342 mg/kg) were the second and fourth least toxic, in female mice they were the two least toxic (LD_{50}s of 1,552 and 1,495 mg/kg, respectively). Although the order of toxicity varies, 2,3-DNT, 2,4-DNT and the ADNTs are less toxic than TNT, and the other isomers are more toxic, in both sexes.

C. Toxic Effects

As with the nitrotoluenes tested earlier, the toxic effects seemed to be on the central nervous system: inactivity, unconsciousness and (in rats given ADNTs) a later hyperexcitability and failure to groom. Most deaths occurred within 24 hr of dosing, but the later deaths in rats and mice and the longer lasting effects in rats suggest the presence of two toxic mechanisms: first, a direct depression leading to unconsciousness which deepened into respiratory paralysis; secondly, a slower, less well defined action producing the motor excitability and failure to groom. This latter phase may be related to the convulsions seen with some nitrotoluene compounds, such as TNT and 3,4-DNT.[1/]

Mice given either 2-ADNT or 4-ADNT and rats given 2-ADNT produced urine of a redder color than the administered compound. This red shift suggests that the metabolism of the compounds affected the substituents on the aromatic ring, probably reducing the nitro groups to amines, thus changing the chromophore.

D. Primary Skin and Eye Irritation

All three compounds tested here (3,5-DNT and the ADNTs) produced less irritation to intact and abraded rabbit skin than the vehicle control. All three were non-irritating to rabbit eyes. These results are similar to those of the other compounds tested.

E. Dermal Sensitivity

None of these three compounds produced dermal sensitization in guinea pigs.

F. Disposition and Metabolism

Qualitatively, the disposition and metabolism of 3,5-DNT and 4-ADNT were much like those reported earlier.[1] The compounds were well absorbed in 24 hr after oral dosing. The extent of absorption of 3,5-DNT was similar to that of TNT. 4-ADNT was less well absorbed but was similar to 2,3-DNT and 2,6-DNT. Both 3,5-DNT and 4-ADNT were widely distributed in various tissues and slightly concentrated by the liver and kidneys.

A majority of the administered 3,5-DNT appeared in the urine in 24 hr, but none of it chromatographed as 3,5-DNT itself. More 4-ADNT-derived radioactivity appeared in the feces than in the urine; the radioactivity in the urine apparently included no 4-ADNT. Therefore, both compounds were metabolized extensively in the body. The aromatic ring apparently remained intact; negligible radioactivity appeared in the expired air.

G. Ames Tests

The munitions compounds studied in this investigation are either nitroaromatics or nitroaliphatics, with the exception of white phosphorus. All of the nitroaromatics studied appeared to be mutagenic to Salmonella typhimurium. Many recent studies have shown that, in general, nitroaromatics exhibit a high degree of mutagenic activity in the Salmonella/microsome plate test.[14-18] These nitroaromatics are thought to exhibit a high degree of mutagen activity to microbial test systems because of their activation by nitroreductase(s) and other metabolizing enzymes[15] endogenous to the microbial tester strains. Furthermore, liver homogenates have recently been shown to activate nitroaromatic compounds to mutagens.[19] In light of these considerations, it appears that TNT and the six DNT isomers should be considered as potentially mutagenic and possibly carcinogenic.

The other class of munitions compounds showing mutagenic activity were the nitroglycerins. TNG is metabolized by mammals to inorganic nitrite.[20,21] Hodgson et al.,[22] have recently reported that the DNGs and MNGs are also metabolized by denitration. It has been shown that inorganic nitrite will react with endogenous primary and secondary amines to form highly mutagenic and carcinogenic nitrosamines.[23] Since the major metabolite of the nitroglycerins is inorganic nitrite, this class of compounds should also be considered as potential mutagens and/or carcinogens.

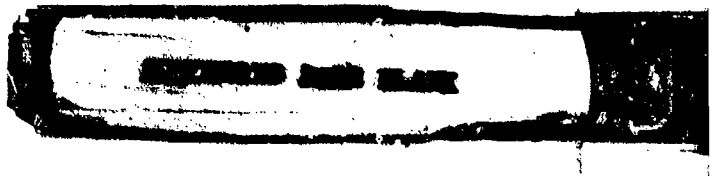
The results of this investigation suggest that several common munitions compounds and chemical intermediates are mutagenic. The tester strains used in this study are currently used in many laboratories as a primary screen of compounds for potential carcinogenic activity. However, the limitations of the Salmonella/microsome plate test should be fully understood before any conclusions can be made on these compounds. A compound demonstrating a positive response in the Salmonella/microsome plate test may not, in fact, be carcinogenic or even mutagenic in mammalian systems. The Salmonella typhimurium tester strains used in this study have been reported to be unusually sensitive for the detection of nitro-containing mutagens and carcinogens.[16] Since many of the compounds did not require metabolic activation or were inactivated in the presence of S-9 microsomal fraction, it is quite possible that the bacteria may contain nitroreductase(s) not found in mammals which result in a false positive in the Salmonella/microsome plate test. Two of the compounds, 2,4-DNT and TNG, displaying mutagenic activity in this study are currently being tested for carcinogenic activity in animals and preliminary findings suggest that 2,4-DNT is carcinogenic to rats.[24]

V. CONCLUSIONS

The acute oral toxicities of all the nitrotoluenes tested are generally similar. 3,5-DNT is the most toxic (LD_{50}s from 216-611 mg/kg) and 2-ADNT the least toxic (LD_{50}s from 1,394-2,240 mg/kg). Acute toxic effects are primarily on the central nervous system: depression, sometimes with hyperreflexia or convulsions. After some compounds, the animals excreted brightly colored urine, of the color of the compound or of a longer wavelength. Only 2,5-DNT was a moderate skin irritant; the others were nonirritating to rabbit eyes. TNT was moderately sensitizing to guinea pigs, 2,6-DNT mildly sensitizing, the others nonsensitizing.

All these nitrotoluenes were fairly well absorbed and widely distributed by rats. They were concentrated in the liver and kidneys. They were extensively metabolized, presumably in the liver, and excreted in the urine.

Ames tests of various munitions compounds found that TNM, TNT, 2,4-DNT, 2,5-DNT and 1,2-DNG were potential mutagens, active at 10 to 30 ug/plate. The other nitrotoluenes tested (2,3-DNT, 2,6-DNT, 3,4-DNT and 3,5-DNT) and TNG and 2-MNG were weakly mutagenic. 1,3-DNG, 1-MNG, NC and WP were not mutagenic in this test.



VI. CORRIGENDA TO REPORT NO. 1

The following typographical errors have been noted in Progress Report No. 1,[1/] and should be corrected.

Page vii, line 3: The parenthetical phrase should read: (1,2-DNG and 1,3-DNG).

Page 3, line 19: Section "b" heading should read: 1,3-DNG:

Page 15, line 28: The reference should be to Figure 7.

Page 31, Table 2: The LD_{50} for females for 1,2-DNG, should have no decimal point, and should read: 1,423 $\pm$ 176.

REFERENCES

1. Lee, C. C., J. V. Dilley, J. R. Hodgson, D. N. Roberts, D. O. Helton, and W. J. Wiegard, Mammalian Toxicity of Munition Compounds: Phase I. Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization, and Disposition and Metabolism, USAMRDC Contract No. DAMD-17-74-C-4073, Report No. 1:1-100, July 2[illegible] 1975, NTIS Report No. AD-B011,150.

2a. Ames, B. N., J. McCann, and E. Yamasaki, Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test, Mut. Teg.31:347-364, 1975.

2b. McCann, J., Mutagenesis, Carcinogenesis, and the Salmonella Test, Chemtech., 6:682-687 (1976).

3. Ellis, H. V., III, J. J. Kowalski, J. R. Hodgson, J. C. Bhandari, J. L. Sanyer, T. W. Reddig, J. L. Minor, and C. C. Lee, Mammalian Toxicity of Munition Compounds: Phase II. Effects of Multiple Doses. Part IV. Nitrocellulose, USAMRDC Contract No. DAMD-17-74-C-4073, Report No. 5:1-96, September 7, 1976. NTIS Report No. AD-A062,015.

4. Helton, D. O., Chemical and Physical Characterization of Nitrocellulose Fines, USAMRDC Contact No. DAMD-17-74-C-4073, Special Report: 1-23, June 7, 1976.

5. Morton, R. A., and A. McGookin, Absorption Spectra of Nitrotoluidines and Related Substances, J. Chem. Soc., 1934:901-911.

6. Finney, D. J., Probit Analysis, Cabridge University Press, 1971.

7. Consumer Products Safety Commission, Code of Federal Regulations Title 16 - Commercial Practices, Part 1500.41, "Method of Testing Primary Irritant Substances" and Part 1500.42, "Test for Eye Irritants," 1975.

8. Magnusson, B., and Albert M. Kligman, The Identification of Contact Allergens by Animal Assay: The Guinea Pig Maximization Test, J. Invest. Derm., 52:268-276, 1969.

9. Roth, L. J., E. Leiter, J. R. Hogness, and W. F. Langham, Studies on the Metabolism of Radioactive Nicotinic Acid and Nicotinamide in Mice, J. Biol. Chem., 176:249-257, 1948.

[illegible] PAGE BLANK

10. DiCarlo, F. J., Malcolm C. Crew, Lloyd J. Haynes, Myriam D. Melgar, and Rosemarie L. Gala, The Absorption and Biotransformation of Glyceryl Trinitrate-1,3-^{14}C by Rats, Biochem. Pharmacol., 17:2179-2183, 1968.

11. Needleman, P., D. J. Blehm, A. B. Harkey, E. M, Johnson, and S. Lang, The Metabolic Pathway in the Degradation of Glyceryl Trinitrate, J. Pharmacol. Exp. Ther., 179:347-353, 1971.

12. Conklin, Cornelius, and Frank Pristera, Preparation and Physical Properties of Di- and Trinitrotoluene Isomers, Feltman Research and Engineering Laboratories, Picatinny Arsenal, Dover, New Jersey, Technical Report 2525, Army Project 5A04-10-006, 1958.

13. Mathias, A., and D. Taylor, The Analysis of Dinitrotoluene Isomer Mixtures by Nuclear Magnetic Resonance, Anal. Chim. Acta, 35:376-382, 1966.

14. Brown, J. P., and R. J. Brown, Mutagenesis by 9,10-Anthraquinone Derivatives and Related Compounds in Salmonella typhimurium, Mutation Res., 40:203-224, 1976.

15. Lindmark, D. G., and M. Meuller, Antitrichomonad Action, Mutagenicity and Reduction of Metronidazole and Other Nitroimidazoles, Antimicrob. Ag. Chemother., 10:476-482, 1976.

16. McCann, J., E. Choi, E. Yamasaki, and B. N. Ames, Detection of Carcinogens as Mutagens in the Salmonella/Microsome Test: Assay of 300 Chemicals, Proc. Nat. Acad. Sci., 72:5135-5139, 1975.

17. Wang, C. Y., K. Muraoka, and G. T. Bryan, Mutagenicity of Nitrofurans, Nitrothiophenes, Nitropyrroles, Nitromidazole, Aminothiophenes, and Aminothiazoles in Salmonella typhimurium, Cancer Res., 34:3611-3617, 1975.

18. Won, W. D., L. H. DiSalvo, and J. Ng, Toxicity and Mutagenicity of 2,4,6-Trinitrotoluene and Its Microbial Metabolites, Appl. Environ. Microbiol., 31:576-580, 1976.

19. Rosenkranz, H. S., and W. T. Speck, Mutagenicity of Metronidazole: Activation by Mammalian Liver Microsomes, Biochem. Biophys. Res. Commun., 66:520-525, 1975.

20. Hay, M., The Chemical Nature and Physiological Action of Nitroglycerine, Practitioner, 30:42-54, 1883.

21. Oberst, F. W., and F. H. Snyder, Studies on Nitrate Esters, I. Nitrite Producing Systems on Rabbit Tissues, J. Pharmacol. Exp. Ther., 93: 444-450, 1948.

22. Hodgson, J. R., J. P. Glennon, J. C. Dacre, and C. C. Lee, Metabolism and Disposition of Isomers of Dinitro- and Mononitroglycerol in the Rat, Toxicol. Appl. Pharmacol., 40:65-70, 1977.

23. Sander, J., F. Schweinberg and H. P. Menz, Untersuchungen ueber die Entstehung Cancerogenes Nitrosamine im Magen, Hoppe-Seylers Z. Physiol. Chem., 349:1691-1697, 1968.

24. Ellis, H. V., III, C. B. Hong, J. C. Dacre, and C. C. Lee, Chronic Toxicity of 2,4-Dinitrotoluene in the Rat, Toxicol. Appl. Pharmacol., 45:245-246, 1978.

DISTRIBUTION LIST

No. of Copies	
25	Commander U.S. Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBG-E Fort Detrick, Frederick, MD 21701
4	HQDA (SGRD-SI) Fort Detrick Frederick, MD 21701
12	Defense Documentation Center (DDC) ATTN: DDC-DCA Cameron Station Alexandria, VA 22314
1	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1	Superintendent Academy of Health Sciences, U.S. Army ATTN: AHS-COM Fort Sam Houston, TX 78234